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QUANTITATIVE STUDIES ON THE FIXATION RATIO "COMPLEMENT : ANTIBODY" IN COMPLEMENT-FIXATION TESTS WITH SYPHILITIC SERUM

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Kaup and Kretschmer (1917) were the first to introduce a quantitative evaluation of syphilitic serum by adding varying amounts of complement (C′) to a constant antigen-antibody mixture. Later, especially in the past decade, some important papers were published in which the respective relations between antibody, antigen and C′ were studied in different ways (Rice, 1948; Mayer, Osler, Bier, and Heidelberger, 1948; Bier, Siqueira, and Furlanetto, 1955; de Almeida, 1956; Osler and Knipp, 1957).

This paper is a further contribution to previous studies (Ruge, 1954), in which I tried to demonstrate that the fixation ratio between antibody and C′ would depend on the law of mass action, using the technique of Kaup and Kretschmer adapted to modern requirements.

This relation has already been established for syphilitic sera by de Almeida, Silverstein, and Maltaner (1952), who employed varying amounts of C′ against constant quantities of antibody-antigen complex and *vice versa*, using the methods of Wadsworth, Maltaner, and Maltaner (1938), and Wallace, Osler, and Mayer (1950).

In the present paper the previous trials have been completed and enlarged in as much as each serum was titrated to the end-point and Pallida Antigen† was used in addition to cardiolipin.

In this way, it was possible to study the fixation ratio antibody: C′ for both antilipoidal and antitreponemal antibodies.

Materials and Methods

Sera.—The sera used in these investigations were drawn from known syphilitics. The diagnosis had been confirmed by clinical, darkfield, and serological examination, *i.e.* S.T.S., *T. pallida* reaction, and the T.P.I. test. All the sera were kept at +4°C., and all examinations were performed as quickly as possible to avoid unnecessary manipulations.

Generally, the usual evaluation of each serum was performed on the first day, and the more detailed studies were carried out on the second day (see below).

Heterophil Antibodies.—These were removed by adding concentrated sheep cells (U.S. Publ. Hlth Service, 1959).

Absorption of Antilipoidal Antibodies.—Precipitated V.D.R.L. antigen was used according to the method of Hardy and Nell (1955) to absorb the antilipoidal serum antibodies. If complete absorption was not obtained in the first instance, the procedure was repeated.

Absorption of Antitreponemal Antibodies.—Since the attempts to absorb antitreponemal antibodies had a poor result, and too great a part of the treponemal suspension had to be employed, these absorption trials were stopped. Therefore, the sera could only be deprived of their antilipoidal antibodies. Moreover, this method of absorbing a single antibody complex proved to be sufficient.

Tests.—After these preparations, the tests were carried out as follows:

Sera diluted from 1 : 2·5 to 1 : 640 were added in the first row with that amount of complement which exactly produced complete (sparkling) haemolysis, *i.e.* "one unit". The following rows, however, received 2, 4, 8, . . "units" of C′ = 2, 3, 4, . . log. units. To avoid too large doses of C′, concentrated C′ was used, *i.e.* 0·2 ml. containing 2, 4, 8, . . . "units", respectively.

Pallida Reaction.—Since the Pallida reaction was set up differently from that with cardiolipin, the respective amounts of C′ had to be added in a different form. Here,

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the percentage of C′ needed to obtain sparkling haemolysis, was used as a starting point. If, for instance, 10 per cent. C′ was found suitable, the following dilutions were made up with saline containing 12·5, 15, 17·5, 20 per cent. C′.

This means that every successive dilution contained 1 × 25 per cent., 2 × 25 per cent., 3 × 25 per cent., ...C′ more than the standard dilution. An example of the evaluation of such a serum is shown in Table I.

In this way, twenty sera were titrated with cardiolipin and eighteen of them were tested with Pallida Antigen, (*a*) without absorption and (*b*) after absorption with V.D.R.L. antigen.

Dilutions.—These were made with ordinary saline, except in the Kolmer test, in which Kolmer saline solution was employed.

The mathematical calculations were based on the method of least squares. The end titre of every row was separately estimated as 100 = log 2·00, and all mathematical values were coordinated with the end titres in question.

Results and Discussion

The results were divided into four groups:

(*a*) Cardiolipin,

(*b*) Pallida Antigen without absorption,

(*c*) Pallida Antigen after absorption,

(*d*) *b* + *c* combined.

The original titres covered a range from 1:20 + + + + to 1 : 640 + +. Since only a few sera were met with in each titre group, the results of all titrations were combined after careful evaluation of each serum.

Generally, the titres with Pallida Antigen remained after absorption at the same level as before; one step above or below the original titre was not considered to be of importance.

This behaviour of the Pallida Antigen supports the assumption of Faure and Pillot (1960) that the Reiter antigens are rather inert concerning their lipoidal part; these, therefore, will play no practical role in sero-diagnosis.

The regression of the titres was not always as equal as was to be expected. Sometimes, the "jumps" were rather remarkable, amounting to several dilution steps, falling for instance from 1:320 + + + + to 1 : 40 + + in the next dilution. On the other hand, the same end-titres were observed in two different rows, although a double quantity of C′ had been added. In the long run, however, all these minor discrepancies were compensated for, and a rather steady decrease of the titres took place.

The evaluation of the cardiolipin end-titres did not require more than 5 (1 + 4) "log. units" of C′ (maximum titre 1 : 640 + +), whereas the Pallida end-titres did not come down to zero until 6 (1 + 5) "25 per cent. units" were consumed (maximum titre 1 : 640 + +). This different behaviour is apparently due to the distinct techniques which were employed in the two complement-fixation tests. From the mathematical point of view, however, no difference respecting the fixation ratio antibody : C′ could be discovered.

The logarithmic values obtained after each additional "C′ unit" are presented in Table II (opposite).

All the values of Table II are arranged as abscissa (antibody) and coordinate (C′ units), respectively. This means that an imaginary line plotted from the "antibody starting point" (log 2·00) to the coordinate (log C′) would fit fairly closely the different groups of points which were observed and calculated from the respective values of titre regression, *i.e.* altogether eight cardiolipin and thirty Pallida-R values, after the addition of 2, 3, 4, . . . log C′ units. For simplicity, only the values for cardiolipin (A) and Pallida combined (D) are given in the Figure (opposite).

On the whole, a good agreement between experimental investigation and mathematical calculation can be demonstrated. More meticulous trials will possibly prove that a still closer approximation may be realized, but eventually diluting factors may lead to some discrepancy.

TABLE I

SERUM DILUTION 0·2 ml., ANTIGEN 0·2 ml., AMBOCEPTOR + SHEEP CELLS 0·4 ml., LOG C′ UNITS OR C′ PER CENT.

Row	1st ..	1 : 2·5+	1 : 5+	1 : 10+	1 : 20+	1 : 40+	1 : 80+	1 : 160+	1 unit	10·0 per cent.
	2nd ..	1 : 2·5+	1 : 5+	1 : 10+	1 : 20+	1 : 40+	1 : 80– –	1 : 160– –	2 units	12·5 per cent.
	3rd ..	1 : 2·5+	1 : 5+	1 : 10±	1 : 20– –	1 : 40– –	....		3 units	15·0 per cent.
	4th ..	1 : 2·5+	1 : 5– –	1 : 10– –	1 : 20– –	.....			4 units	17·5 per cent.
	5th ..	1 : 2·5– –	1 : 5– –	1 : 10– –	.....				5 units	20·0 per cent.

TABLE II

LOG TITRES OBTAINED WITH INCREASING DOSES OF COMPLEMENT

C′ Units	(A) Cardiolipin			(B) Pallida Before			(C) Pallida After			(D) B and C combined		
	Obs.	Calc.	M	Obs.	Calc.	M	Obs.	Calc.	M	Obs.	Calc.	M
1	2·02	2·00	±0·01	2·06	2·00	±0·02	2·03	2·00	±0·02	2·04	2·00	±0·01
2	1·77	1·72	±0·04	1·80	1·75	±0·05	1·77	1·69	±0·03	1·78	1·75	±0·03
3	1·46	1·37	±0·07	1·41	1·35	±0·06	1·42	1·33	±0·08	1·42	1·35	±0·05
4	1·02	0·90	±0·08	1·10	0·91	±0·10	1·19	1·09	±0·08	1·15	1·00	±0·07
5	0·59	0·35	±0·11	0·65	0·54	±0·09	0·80	0·69	±0·08	0·72	0·61	±0·06
6				0·31	0·24	±0·07	0·34	0·28	±0·08	0·32	0·26	±0·05

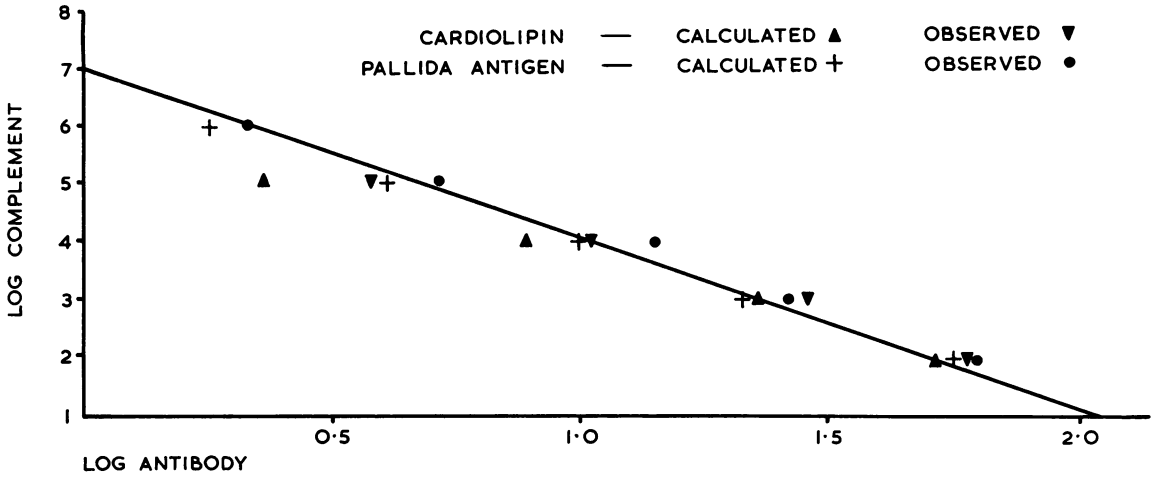

Figure: Distribution of observed and calculated values for Cardiolipin (A) and Pallida Antigen (D) on logarithmic scale (cf. Table II).

The following equations were prepared:

Cardiolipin: y (log C′) = 4·43 − 2·23 × (log antibody)
Pallida not absorbed:
y (log C′) = 4·28 − 2·11 × (log antibody)
Pallida absorbed:
y (log C′) = 4·28 − 2·10 × (log antibody)
Pallida combined:
y (log C′) = 4·42 − 2·21 × (log antibody)

These results demonstrate the dependence of the complement-fixation tests for syphilis on the law of mass action, in as much as two parts antibody are bound by one part C′. Besides, both antibodies react in the same way: *i.e.* the fixation of different antibodies—antilipoidal and/or antitreponemal—takes place for both antibodies in the same proportion. Thus it does not matter whether the experiments are carried out on sera exhibiting a high (>1 : 80) or a low (<1 : 40) titre.

It has also been ascertained that the removal of antilipoidal antibodies does not impair the reactivity of the serum towards antitreponemal protein-antibodies. On the other hand, the influence of antitreponemal antigens on antilipoidal antibodies remains to be evaluated.

Summary

The antilipoidal and antibody contents of twenty syphilitic sera have been quantitatively evaluated by adding increasing amounts of complement. These investigations were carried out with cardiolipin antigen.

Eighteen of these sera have also been examined with Pallida Antigen* for antitreponemal antibodies, with and without previous absorption of the antilipoidal antibodies.

* Pallida-Antigen Promonta, Hamburg 26.

It could be ascertained that the fixation ratio antibody : complement in all experiments was 2 : 1, *i.e.* two parts antibody—both antilipoidal and antitreponemal—are bound by one part complement, according to the law of mass action.

It appears that this behaviour extends to all titres of serum, and that probably some discrepancies between "observed" and "calculated" values—the calculations being performed according to the method of least squares—may be explained by the dilution factor and technical difficulties.

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Etudes quantitatives sur le rapport fixatif "Complément: Anticorps" dans les tests fixant complément contre sérum syphilitique.

RÉSUMÉ

Le contenu quantitatif des anticorps antilipoïdes ds vingt sérums syphilitiques a éte évalué en ajoutant dee quantités de complément de plus en plus grandes. Ces épreuves ont été faites avec l'antigène cardiolipine.

18 de ces sérums ont été examinés en plus avec "Pallida-Antigen"* pour les anticorps antitréponèmes, avant et après l'absorption des anticorps antilipoïdes.

Dans chaque épreuve le rapport fixatif "Complément: Anticorps" était 2 : 1; c'est à dire que deux parties d'anticorps (soit antilipoïde ou antitréponème) sont fixées par une partie de complément, selon la loi d'action en masse.

Il semble que tous les titres de sérum se portent du même façon, et que quelques différences entre les taux "observés" et "calculés" (les calculs faits par la méthode de carré minimum) peuvent être expliqués par le facteur de la dilution et par les obstacles technologiques.